Neuron, Vol. 33, 673–675, February 28, 2002, Copyright ©2002 by Cell Press

Genetic Models Meet Trophic Mechanisms: EGF Family Members Are Gliatrophins in *Drosophila*

Minireview

Gad Beck and Mike Fainzilber[1]
Department of Biological Chemistry
Weizmann Institute of Science
76100 Rehovot
Israel

Trophic survival mechanisms are crucial for the determination of cell numbers in the developing vertebrate nervous system, but important neurotrophic factor families such as the neurotrophins have not yet been found in either *Drosophila* or *C. elegans*. Two independent studies on distinct glial populations in *Drosophila* have now shown that their survival is regulated by EGF family members secreted by adjacent neurons. Fly genetics thus promises new insights on trophic signaling mechanisms and confirms that trophic regulation of cell survival is an evolutionarily ancient mechanism for building the nervous system.

Cell numbers in the vertebrate nervous system are determined by proliferation of precursors early in development, followed by death of excess cells at later stages. The extent of the latter process is determined by survival factors produced in limiting amounts by target or adjacent tissues, thereby matching cell numbers to the trophic (survival) factor-production capacity of the supporting tissue. The main focus of research on trophic factors determining neuronal survival are the NGF family of neurotrophins and the GDNF family ligands (GFLs). Surprisingly, despite their undoubted importance in mammalian development, both the neurotrophins and the GFLs have not been found in the fly and nematode genomes (Jaaro et al., 2001). Hence, research on trophic mechanisms has been constrained by the lack of an invertebrate genetic model. Although *C. elegans* is thought to be mainly "hard-wired," the lack of obvious trophic factors in *Drosophila* has been puzzling. Do insects get by without trophic mechanisms, or do they utilize other molecules as trophic factors? If trophic factors are at work in insects, why haven't they turned up in the numerous genetic screens done in the fly?

Previous studies in *Drosophila* have provided tantalizing hints for trophic survival mechanisms. Most notably, the EGF receptor/RAS/MAPK pathway has been implicated in the control of both proliferation and survival of cells in the developing eye. Mitotic signaling through the EGFR increases the number of progenitor cells during the last cell cycle of eye development, and subsequent EGFR signaling is required to maintain cell survival (reviewed in Baker, 2001). In the nervous system, longitudinal connective neurons were shown to undergo programmed cell death following ablation of adjacent glial cells (Booth et al., 2000). Another study demonstrated that pioneer neurons maintain the survival of longitudinal glia (Kinrade et al., 2001). However, there was no molecular identification of a candidate factor(s) underlying these phenomena, and the possibility that they exemplified bona fide trophic interactions remained unproven. Two new studies published within months of each other in *Developmental Cell* now show that the neuregulin homolog Vein maintains survival of a subpopulation of longitudinal glia (Hidalgo et al., 2001), while the TGFα homolog Spitz maintains survival of midline glia (Bergmann et al., 2002). In both cases, the trophic ligands are secreted by adjacent axons at concentrations sufficient for the survival of only a subset of the target glial population. Thus, two different members of the EGF family are shown to be gliatrophins in *Drosophila*.

Hidalgo et al. (2001) and Bergmann et al. (2002) converged on the same basic mechanism from two different directions. Hidalgo et al. (2001) followed on from their recent analysis of longitudinal glial survival in the fly (Kinrade et al., 2001). The longitudinal glia (LG) move medially after their birth to contact the pioneering axons of the longitudinal fascicles. They then migrate together with the axonal growth cones, eventually extending on and ensheathing the longitudinal nerve tracts (Jones, 2001). Once in contact with the axons, Kinrade et al. (2001) found that the LG become dependent on them for survival. Since Vein, the fly homolog of neuregulin, is expressed in pioneer axons, Hidalgo et al. (2001) tested whether it regulated LG survival. LG were found to be present in excess before axon-glia contact, with onset of apoptosis in doomed cells shortly after contact was established. Apoptosis of LG was increased in *vein* null mutants. In order to assess if this was due to Vein expressed by the axons, RNAi was specifically targeted to the pioneer neurons. Loss of LG was still observed in this experimental situation, albeit at much lower levels, suggesting that Vein might not be the only survival factor for LG. Dominant-negative DER (*Drosophila* EGF Receptor) expression in the glia also increased apoptosis. Finally, expression of a *vein* transgene in pioneer neurons rescued glial cells. Taken together, the data suggest that Vein secreted by the neurons provides part of the trophic support required by the LG.

Neuronal ablation or *Ras* null mutants exhibit more severe glial phenotypes than seen in the *vein* mutants, suggesting that additional factors may regulate glial survival. An additional candidate ligand for LG survival is the TGFα homolog Spitz, since LG loss increases in *vein*;*spitz* double mutants. Other signaling pathways may also be involved, since DER is not activated in all the LG examined. Additional pathways that might be integrated to determine glial survival include, for example, the FGF receptor homolog *heartless*, which is also expressed in LG. Other candidate trophic receptors are known in the fly, most prominently a well-conserved Ret homolog (Sugaya et al., 1994). Although a recognizable Ret ligand is yet to be found in *Drosophila*, indirect routes of Ret activation were recently suggested to be of physiological relevance in mammals (Tsui-Pierchala et al., 2002). Hopefully there will now be renewed impetus for analysis of mutant allelles of Ret and other candidate trophic RTKs in *Drosophila*.

Bergmann et al. (2002) set out to assess the physiolog-

[1] Correspondence: mike.fainzilber@weizmann.ac.il

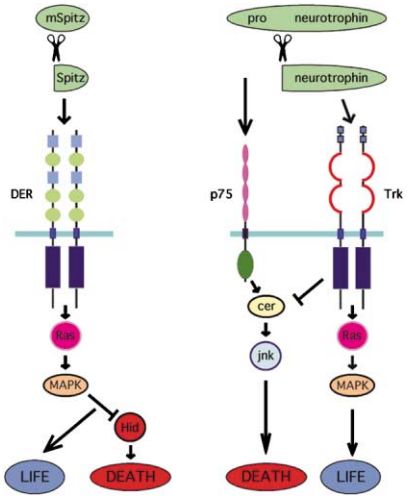

Figure 1. Gliatrophin Signaling in *Drosophila* versus Neurotrophin Signaling in Vertebrates—Similar Principles Exemplified by Different Molecules

Both fly Spitz and mammalian neurotrophins are synthesized as precursor forms. Membrane spitz (mSpitz) is inactive in signaling, thus allowing a default Hid-induced apoptosis pathway to proceed. Proneurotrophins preferentially activate an apoptotic pathway via the p75 receptor. Upon cleavage of mSpitz, soluble Spitz activates the DER pathway, which promotes survival via a Ras-MAPK inhibition of Hid. Upon cleavage of proneurotrophins, mature neurotrophins preferentially activate Trk receptors, which promote survival both via MAPK signaling and by inhibiting the apoptotic signal emanating from p75.

ical significance of apoptosis mediated by *hid*, a cell death inducer that is inhibited upon direct phosphorylation by MAPK (Bergmann et al., 1998). Importantly, Bergmann et al. (2002) chose to analyze the effects of Spitz on *Drosophila* midline glia (MG), which undergo stochastic and extensive cell death during development. This prescient choice of an experimental system was rewarded with clear-cut results, allowing the complete delineation of a trophic survival pathway in the fly (see Figure 1). Bergmann et al. (2002) first showed that MG survival is directly correlated with MAPK activity levels and requires direct phosphorylation of HID by MAPK. In order to examine a role for DER in this process, dominant-negative DER was expressed in the MG after their generation, severely compromising their subsequent survival. A large reduction in MG numbers in *spitz* null mutants indicated that *spitz* was the likely candidate ligand. Spatial and temporal specificity in Spitz signaling is determined by regulated proteolysis of its ubiquitously expressed membrane-linked precursor (mSpitz). Expression of mSpitz in the MG of spitz embryos does not lead to autocrine rescue, whereas mSpitz transgenes targeted to neurons efficiently rescued adjacent MG (Bergmann et al., 2002). MG are rescued from apoptosis in *spitz*;*hid* double mutants, suggesting that MG survival is regulated by a Spitz signal that suppresses Hid. Moreover, overexpression of activated Spitz leads to rescue of additional MG, indicating that physiological levels of Spitz are limiting, as one would expect from a trophic factor. These data define a complete trophic signaling cascade from the extracellular ligand Spitz, through the cell surface EGFR and the RAS/MAP kinase pathway to suppression of a default death pathway dependent on the pro-apoptotic protein Hid (see Figure 1). The mitochondrial pro-apoptotic protein Smac/DIABLO is a structural and functional homolog of Hid (Srinivasula et al., 2001); therefore, homologs for all components of this trophic pathway exist in vertebrates. Direct predictions arising from the work of Bergmann et al. (2002) on the regulation of apoptotic signaling cascades in vertebrate systems can now be tested.

In addition to the molecular parallels between mammalian and *Drosophila* systems outlined above, there are striking physiological similarities in glial survival mechanisms. The effects of the fly EGF family members described by Hidalgo et al. (2001) and Bergmann et al. (2002) are highly reminiscent of the survival-promoting effects of axonal-derived neuregulin-1 (NRG-1) on Schwann cells in the mammalian nervous system (reviewed by Lemke, 2001). As seen for Vein and Spitz in the fly, NRG-1 plays assorted roles at multiple stages of Schwann cell development, regulating differentiation, proliferation, and finally survival to match the number of Schwann cells to the number of NRG-1-producing neurons. As might be expected from the larger cell numbers and diversity in mammals, there is a corresponding diversification of ligands and receptors. NRG-1 is produced in multiple isoforms by alternative splicing, and these isoforms interact with at least three EGFR-related ErbB receptors (Lemke, 2001). Moreover, other ligand-receptor systems may affect Schwann cell survival independently of the NRG-1-ErbB pathway. For example, recent results suggest that NGF acting via the p75 neurotrophin receptor can induce either death or survival of Schwann cells, depending on the levels of RIP2, an intracellular interactor (Khursigara et al., 2001). Apoptotic signaling of p75 in Schwann cells may parallel the default Hid-induced death in fly glia, and it will be interesting to examine the possible involvement of Smac/DIABLO in the mammalian system. Conversely, it may be rewarding to examine if additional signaling pathways described in mammalian glia (e.g., Parkinson et al., 2001) play a role alongside EGFR signaling in regulation of glial survival in *Drosophila*.

Given the extensive research on *Drosophila* EGF receptor signaling, why have survival-promoting effects of EGF family ligands in the nervous system not been emphasized previously? The multitude of functions for DER and its ligands during development provide part of the answer, as almost any null mutant for a component of this pathway has drastic effects in early development of the fly (Schweitzer and Shilo, 1997) before trophic mechanisms are required or apparent in development. Even the use of more selective approaches such as temperature-sensitive alleles (Raz and Shilo, 1992) or enhancer traps (Klambt et al., 1991) did not allow discrimination between effects on differentiation versus effects on survival of the MG cells. Indeed, both Hidalgo et al. (2001) and Bergmann et al. (2002) required spatially and temporally targeted loss-of-function techniques (RNAi, dominant-negative transgenes) to provide strong experimental support for their trophic models. This suggests that additional trophic pathways might be uncovered in the fly by application of high-resolution techniques such as neuron-specific mosaic analysis (Lee

and Luo, 2001). It is interesting to note that trophic effects of EGF family members may have been similarly overlooked in vertebrates. Although trophic support of peripheral glia by axon-derived neuregulins is well-established in mammals (Lemke, 2001), survival-promoting effects of the *spitz* homolog TGFα have not been emphasized. TGFα has a veritable multitude of reported effects on different cells of the CNS, further complicated by a lack of coherence between the phenotypes of knockout versus overexpressor transgenic mice for this ligand (Junier, 2000). The rather drastic nonneuronal phenotype of EGFR knockout mice also hampers the analysis of trophic roles for this signaling pathway in the nervous system. Nonetheless, a very recent paper presents intriguing evidence for an autocrine role of TGFα in motoneuron survival (Boillee et al., 2001). The relative simplicity of the *Drosophila* nervous system and the power of fly genetics can now be employed to shed light on possible trophic roles of TGFα in neurons.

Are survival-promoting roles of EGF family ligands likely to be phylogenetically widespread? EGF family ligands have been found in nearly all multicellular organisms examined to date, including LIN-3 in the nematode *C. elegans* and L-EGF in the mollusk *Lymnaea stagnalis* (Hermann et al., 2000). Although survival-promoting activities of these molecules have not yet been reported in the nervous system, a number of accessory trophic roles have been postulated for the molluscan EGF, including support of neurite outgrowth (Hermann et al., 2000). It is, therefore, quite likely that the EGF family represents one of the earliest and most widespread examples of trophic factors and that the signaling pathway described by Bergmann et al. (2002) is as close as one can get to an ancestral trophic mechanism. Comparing this pathway with the recently updated neurotrophin signaling pathway in vertebrates (Lee et al., 2001), the basic principles are remarkably similar (see Figure 1) despite the differences in the molecular players. The tools of fly genetics have been incredibly useful in many fields of neuroscience and although they are coming late to the trophic factors field, better late than never.

Selected Reading

Baker, N.E. (2001). Semin. Cell Dev. Biol. *12*, 499–507.

Bergmann, A., Agapite, J., McCall, K., and Steller, H. (1998). Cell *95*, 331–341.

Bergmann, A., Tugentman, M., Shilo, B.Z., and Steller, H. (2002). Dev. Cell *2*, 159–170.

Boillee, S., Cadusseau, J., Coulpier, M., Grannec, G., and Junier, M.P. (2001). J. Neurosci. *21*, 7079–7088.

Booth, G.E., Kinrade, E.F., and Hidalgo, A. (2000). Development *127*, 237–244.

Hermann, P.M., van Kesteren, R.E., Wildering, W.C., Painter, S.D., Reno, J.M., Smith, J.S., Kumar, S.B., Geraerts, W.P., Ericsson, L.H., Smit, A.B., et al. (2000). J. Neurosci. *20*, 6355–6364.

Hidalgo, A., Kinrade, E.F., and Georgiou, M. (2001). Dev. Cell *1*, 679–690.

Jaaro, H., Beck, G., Conticello, S.G., and Fainzilber, M. (2001). Trends Neurosci. *24*, 79–85.

Jones, B.W. (2001). Bioessays *23*, 877–887.

Junier, M.P. (2000). Prog. Neurobiol. *62*, 443–473.

Khursigara, G., Bertin, J., Yano, H., Moffett, H., DiStefano, P.S., and Chao, M.V. (2001). J. Neurosci. *21*, 5854–5863.

Kinrade, E.F., Brates, T., Tear, G., and Hidalgo, A. (2001). Development *128*, 207–216.

Klambt, C., Jacobs, J.R., and Goodman, C.S. (1991). Cell *64*, 801–815.

Lee, T., and Luo, L. (2001). Trends Neurosci. *24*, 251–254.

Lee, R., Kermani, P., Teng, K.K., and Hempstead, B.L. (2001). Science *294*, 1945–1948.

Lemke, G. (2001). Annu. Rev. Neurosci. *24*, 87–105.

Parkinson, D.B., Dong, Z., Bunting, H., Whitfield, J., Meier, C., Marie, H., Mirsky, R., and Jessen, K.R. (2001). J. Neurosci. *21*, 8572–8585.

Raz, E., and Shilo, B.Z. (1992). Development *114*, 113–123.

Schweitzer, R., and Shilo, B.Z. (1997). Trends Genet. *13*, 191–196.

Srinivasula, S.M., Hegde, R., Saleh, A., Datta, P., Shiozaki, E., Chai, J., Lee, R.A., Robbins, P.D., Fernandes-Alnemri, T., Shi, Y., and Alnemri, E.S. (2001). Nature *410*, 112–116.

Sugaya, R., Ishimaru, S., Hosoya, T., Saigo, K., and Emori, Y. (1994). Mech. Dev. *45*, 139–145.

Tsui-Pierchala, B.A., Milbrandt, J., and Johnson, E.M. (2002). Neuron *33*, 261–273.